Brazilian Journal of Development

In vitro behavior of *Fusarium* spp. isolates in differents cultivations conditions

Comportamento in vitro de isolados de *Fusarium* spp. em diferentes condições de cultivo

DOI:10.34117/bjdv6n7-655

Recebimento dos originais: 20/06/2020
Aceitação para publicação: 24/07/2020

Rommel dos Santos Siqueira Gomes
Doutor em Agronomia pela Universidade Federal da Paraíba (UFPB/CCA/Campus II)
Instituição: Programa de Pós-graduação em Agronomia na Universidade Federal da Paraíba
Centro de Ciências Agrárias - Campus II
Endereço: Rodovia PB 079, Km 12, Caixa Postal: 66, CEP: 58397-000, Areia-PB, Brasil
E-mail: pratacca@gmail.com

Andréa Celina Ferreira Demartelaere
Doutora em Agronomia pela Universidade Federal da Paraíba (UFPB/CCA/Campus II)
e Professora em Agroecologia
Instituição: Escola Técnica Estadual Senador Jessé Pinto Freire
Endereço: Rua Monsenhor Freitas, 648, Centro, CEP: 59586-000, Parazinho-RN, Brasil
E-mail: andrea_celina@hotmail.com

Wendel Oliveira Maciel
Mestre em Fitopatologia pela Universidade Federal de *Lavras (UFLA)*
Instituição: Programa de Pós-Graduação em Fitopatologia na Universidade Federal de *Lavras*
Endereço: Aquenta Sol, Caixa Postal: 3037, *CEP:* 37200-000, *Lavras*-MG, Brasil
E-mail: wendel.appis@gmail.com

Fábio Júnior Araújo Silva
Doutor em Fitopatologia pela Universidade Federal Rural de Pernambuco (UFRPE)
Instituição: Programa de Pós-Graduação em Fitopatologia na Universidade Federal Rural de Pernambuco
Endereço: Dom Manoel de Medeiros, S/N, Dois Irmãos, CEP: 52171-900, Recife-PE, Brasil
E-mail*:* fabiojr-@hotmail.com

Luciana Cordeiro do Nascimento
Doutora em Fitopatologia pela Universidade Federal Rural de Pernambuco (UFRPE)
e Professora Titular de Fitopatologia
Instituição: Universidade Federal da Paraíba (UFPB/CCA/ Campus II)
Endereço: Rodovia PB: 079, Km: 12, Caixa Postal: 66, CEP: 58.397-000, Areia-PB, Brasil
E-mail: luciana.cordeiro@cca.ufpb.br

Daiane Bernardi
Mestrado em Agronomia pela Universidade Estadual do Oeste do Paraná (UNIOESTE)
Instituição: Programa de Pós-graduação em Agronomia na Universidade Estadual do Oeste do Paraná
Endereço: Rua Pernambuco, 1777, Caixa Postal: 91, CEP: 85960-000, *Marechal Cândido Rondon*-PR, Brasil
E-mail: daiane_ber@gmail.com

Brazilian Journal of Development

Selma dos Santos Feitosa
Doutora em Agronomia pela Universidade Federal da Paraíba (UFPB/CCA/Campus II)
e Professora do CST Agroecologia
Instituição: Instituto Federal de Educação, Ciência e Tecnologia da Paraíba - IFPB, Campus Sousa,
Endereço: Rua Pres. Tancredo Neves, S/N, Jardim Sorrilandia, CEP: 58805-345,
Distrito de São Gonçalo-PB, Brasil
E-mail: selma.feitosa@ifpb.edu.br

Hailson Alves Ferreira Preston
Doutor em Fitopatologia pela Universidade Federal Rural de Pernambuco (UFRPE)
e Professor Adjunto em Fitopatologia
Instituição: Universidade Federal do Rio Grande do Norte (UFRN/EAJ)
Endereço: Rodovia RN 160, Km 03, S/N, CEP: 59280-000, Distrito de Macaíba–RN, Brasil
E-mail: hailson_alves@hotmail.com

ABSTRACT
Ananas comosus (L) Merril var. comosus is the most important fruit segment in Brazil. In Paraíba, the occurrence of fusariosis is associated with humidity, light and temperature, important factors for the reproduction and dissemination of *Fusarium* spp. The aim of the study was to evaluate mycelial growth and sporulation of *F*. spp. under different cultivation conditions. The 10 isolates were grown at different temperatures, luminosities and a Potato Dextrose Agar (PDA) culture medium. Mycelial growth was measured with a ruler graduated in centimeters (cm), at 24-hour intervals, for six days. Conidial production was also quantified with the aid of a Neubauer chamber (x 10^5 conidia/mL). The design was completely randomized in a factorial arrangement (3x3x10) and 15 repetitions. The averages were compared by Scott-Knott ($p \leq 0.05$) in the statistical program R. At temperatures of 15 and 35 °C, in all light regimes, there was no stimulus for the production of mycelial when the ten isolates were evaluated, with the exception of the temperature of 35 °C, in the alternating light regime, which obtained. At a temperature of 25 °C, isolates 5 and 10 stood out from the others in the mycelial growth of *F*. spp., with isolates that produced low and medium mycelium production. For the production of conidia, the temperature of 15 °C, in the three light regimes, a small production of conidia (x 10^5/mL) was observed, at the temperature of 25 °C in the light and continuous dark regimes, there was a greater production of conidia (x 10^5/mL) in isolates 5 and 20 in relation to the other isolates, already in the alternating light regime, all isolates obtained a high production of conidia (x 10^5/mL). At a temperature of 35 °C, with continuous light and dark regimes and alternating light for isolates 1, 2, 3, 4 and 7, they presented an average production, whereas isolates 5, 6, 8, 9 and 19 obtained high productions conidia (x 10^5/mL) of *F.* spp. The recommended culture conditions for mycelial growth and multiplication of *Fusarium* spp. are the alternating light regime and temperature of 25 °C.

Keywords: Fusariosis, Light regime, Mycelial growth, Sporulation, Temperature.

RESUMO
Ananas comosus (L) Merril var. comosus é o segmento frutícola de maior importância no Brasil. Na Paraíba, a ocorrência da fusariose está associada a umidade, luminosidade e temperatura. Esses fatores são importantes para a reprodução e disseminação do Fusarium guttiforme Nirenberg e O'Donnell. O objetivo do estudo foi avaliar o crescimento micelial e esporulação de isolados de F. guttiforme sob diferentes condições de cultivo. O experimento foi conduzido no LAFIT/UFPB/CCA, Areia-PB. Os 10 isolados foram cultivados em temperaturas: 15, 25 e 35 °C, sob regimes: claro e escuro contínuo, luz alternada no meio BDA. O crescimento micelial foi realizado com régua centimetrada (cm), em intervalos de 24 h, por seis dias, a quantificação de conídios em câmara de Newbauer (x 10^5 conídios/mL). O delineamento foi inteiramente casualizado em arranjo fatorial

Brazilian Journal of Development

(3x3x1x10), e 15 repetições. As médias comparadas pelo Scott-Knott ($p \leq 0,05$), programa R®. As temperaturas abaixo de 15 °C, não houve estímulo na produção de micélio e conídios, acima de 30 °C, ocorreu decréscimo, e nas temperaturas 25 a 30 °C, altos índices. As condições de cultivo recomendadas para o crescimento e esporulação de F. guttiforme são luz alternada e temperatura de 25 °C.

Palavras-chave: Crescimento micelial, Esporulação, Fusariose, Regime de luz, Temperatura.

1 INTRODUCTION

The pineapple crop (*Ananas comosus* (L) Merril var. Comosus Coppens & Leal) represents the most important fruit segment in Brazil, currently the state of Paraíba, is the second largest national producer, with a production of 363.330 million fruits, in a harvested area of 60 thousand hectares (IBGE, 2019). However, this production has been decreasing each year and significantly due to the occurrence of fusariosis caused by *Fusarium* spp. (SOUZA *et al*., 2017), also known as resinosis and fungal gummy disease, which is considered a key pineapple disease (AQUIJE *et al.*, 2010).

This pathogen has the ability to infect seedlings, which can cause losses of up to 50%, plants in vegetative development and fruits with losses estimated between 50 to 100%, causing vascular discoloration, lesions in the affected tissues and with exudation of a gummy substance, which makes the quality unfeasible, as well as commercialization (PAULINO *et al*., 2019).

In the state of Paraíba, Brazil, the occurrence of fusariosis in pineapple culture is associated with environmental factors such as humidity, light and temperatures that vary between 25 and 30 °C being important for reproduction and dissemination of the causal agente (GARCIA *et al*., 2015).

However, little is known about the influence of these variations in the environment on the genetic variability of the pathogen that causes serious damage to pineapple growers, as it reduces the quality and productivity of fruits in the different producing regions (TOFOLI *et al.*, 2013).

In addition to the conditions of the development environment, each microorganism requires minimal nutritional needs for its growth and sporulation. Notably, the concentration of nutrients inserted in the culture media, light and temperature can enhance mycelial growth and production of conidia in phytopathogenic fungi in a short period of time (POLTRONIERI *et al.*, 2013).

Silva; Teixeira (2012) evaluating the sporulation and mycelial growth of *Fusarium solani* in different culture media and light regimes, concluded that the greatest mycelial and conidia production occurs through the association of the continuous light regime in the culture medium Potato Dextrose Agar (PDA) for a period of 14 days.

Further research is necessary, as there is difficulty in obtaining sporulant isolates, or even standardizing ideal conditions for the sporulation of phytopathogenic fungi, since this is one of the main problems faced by research groups that aim to identify resistant cultivars (CRUZ *et al.*, 2009).

The effectiveness of management strategies for disease control is clearly dependent on understanding the ecology of the pathogen and its population dynamics. Therefore, studies on variability in fungal populations can be an important research tool (COSTA *et al.*, 2010).

From an evolutionary point of view, determining the genetic variability of populations is essential for the adaptation of the organism under different environmental conditions and from an epidemiological point of view, and will have direct implications for crop management (SOUZA, 2015).

In view of the above, the objective of the study was to evaluate the mycelial growth and sporulation of *Fusarium* spp. under differents cultivations conditions.

2 MATERIALS AND METHODS

The physiological analysis was performed at the Laboratório of Fitopatplogia (LAFIT), belonging to the Centro de Ciências Agrárias of the Universidade Federal of Paraíba, Campus II, Areia-Paraíba, Brazil. *Fusarium* spp. used in the present study came from several pineapple produced in regions the three differents states of Northeast, for example, Paraíba: 1 (Espirito Santo), 2 (Sapé), 3 (Itaporanga), 4 (Guarabira), 6 (Mamanguape), 7 (Mari), 8 and 9 (Rio Tinto), Pernambuco: 5 (Pombos) and Rio Grande do Norte: 10 (Touros).

The collection of pineapple plant material was done in 10 plants at random, which presented symptoms of fusariosis (with lesions in the affected tissues and with exudation of gummy substance), with minimum spacing between plants of 50 m and zigzag sampling according to the methodology described by Demartelaere (2015).

Subsequently, the samples were transported in Kraft paper bags, identified, packed in thermal boxes and transported to LAFIT, where the isolation was done in a culture medium Potato Dextrose Agar (PDA) (200 g potato, 20 g dextrose, 20 g agar, 1000 mL of Sterile Distilled Water (SDW)). The morphological identification was performed through the visualization of structures of the pathogen under microscopy and identification, according to the specialized literature of *F.* spp. in pineapple fruits (BISBY *et al.*, 2006; LESLIE *et al.*, 2006; LIMA *et al.*, 2001).

The 5 mm diameter fragments of pineapple fruits were obtained from ten *F.* spp. isolates, then disinfected by washing in 70% ethanol for thirty seconds, 1% hypochlorite for three minutes and rinsing with sterile distilled water (SDW). Then, the PDA medium was added with the fungicide carbendazin (methyl benzimidazol-2-ylcarbamate) at a dose of 50 mL/100 L, to inhibit the proliferation of contaminating fungi and later, three disks of fungal colonies of the isolates of *F.* spp were incubated with a diameter of 5 mm at room temperature (25 ± 2 °C) for seven days (MENEZES; ASSIS, 2004).

Then, the structures were pigmented with methylene blue, evaluating the texture and consistency of the lower and upper part of the developed colonies, and the microstructures were placed on microscope slides, and visualized under an electron microscope (100x), using immersion oil, according to Nirenberg; O'Donnel (1998). The morphological characteristics of *F*. spp. were confirmed by structures that initially presented white mycelium, changing from pinkish orange to violet.

With the presence of conidia of the macroconidium type (usually with 3 septa) and microconidium (0-1 septum), it presents an obovoid shape of the microconidia in polyphenol, the main characteristic of this phytopathogen, in addition to having erect or prostrate conidiophores and absence of chlamydospores and at most three conidiogenic openings with a greater quantity than monofials (NIRENBERG; O'DONNELL, 1998; O'DONNELL *et al.*, 1998).

After the identification of the ten isolates, a monosporic culture was removed from each isolate. For this, a sporulated culture sample was deposited in a test tube containing 10 mL of sterile distilled water. After stirring, it was subjected to a serial dilution process (4 times). Then, an aliquot containing 1 ml of this diluted suspension was added on the surface of Petri dishes containing agar-water culture medium (20%), which were conditioned for nine hours, and incubated at a constant temperature of 25 °C on light regime.

After this period, the emission of the germ tube of the conidia was found, where a conid was isolated in the field of view of the objective in an electron microscope (100x). This fragment of medium containing the conidium was transferred to a test tube containing PDA culture medium and incubated at a constant temperature of 25 °C and a photoperiod of 12 hours. After 15 days, cultures grown for 9 cm diameter Petri dishes containing PDA medium were grown.

From the monosporic cultures of the isolates, the pathogenicity test was performed, according to the methodology described by Santos *et al.* (2002). As all colonies developed were pathogenic in preliminary tests performed on pineapple fruits, the 10 isolates were selected for the present study.

Mycelial growth was calculated using the mycelial diameter of ten isolates of *F*. spp. under. *Biochemical Oxygen Demand* (B.O.D) conditions, adjusted in different light regimes: continuous light, continuous dark and alternating light (12 hours of light and 12 hours of dark), temperatures of 15, 25 and 35 °C and a half of PDA culture (MENEZES; ASSIS, 2004). The pH of all media was adjusted to 6.0 and then sterilized in an autoclave at 121 °C for 15 minutes.

The evaluations were performed at 24-hour intervals, with a ruler graduated in centimeters (cm), measuring mycelial growth (MG) by the average of two orthogonal axes until reaching the entire Petri dish (80 x 100 mm), which until the sixth day of evaluation, (where measurements were

made every 2 days), according to the adaptation of the methodology described by Oliveira (1991), using the following formula:

$$CM = \frac{C1+ C2 + C3 + C4 + C5}{A2+ A4 + A6 + A8 + A10 + A12}$$

Where C1, C2, C3, C4, C5 are the sum of the growths of the colonies, in relation to the days of avaliations, divided by the number of avaliations that were made with 2, 4, 6, 8, 10 and 12 days (A2, A4, A6, A8, A10 and A12).

Sporulation was obtained from the suspension of conidia of each isolate of *F.* spp., adding 10 mL of SDW to the Petri dishes with the pure colony of the fungus. With the aid of a sterile spatule, the spores were released, then filtered through a double layer of sterile gauze, quantified in a Neubauer chamber, and the suspension was adjusted to 10^5 conidia/mL (MARTELLETO, 1995).

The experimental design used was completely randomized, in a factorial arrangement (3 x 3 x 10), with three light regimes: continuous light, continuous dark and alternating light (12h light and 12h in the dark) and temperatures of 15, 25 and 35 °C and ten isolates of *F.* spp., with 15 replicates.

The analysis of variance was performed to verify the differences in mycelial growth and sporulation in relation to the isolates under the different culture conditions using the F test ($p \leq 0.05$). In this case, the averages were grouped to analyze the isolates and the effects of light regimes, temperatures and the interactions between them on mycelial growth and *F.* spp. The means were compared using the Scott-Knott Test ($p \leq 0.05$) and the analyzes were performed using the R statistical program (R CORE TEAM, 2011).

3 RESULTS AND DISCUSSION

When the continuous light regime was used, at a temperature of 15, 25 and 35 °C, there were significants differences in relation to the mycelial growth of *F.* spp., at 15 °C, the isolates 1 and 2, showing the smallest growths, isolates 3, 4 and 5 showed median growths. The 6 to 10 isolates, on the other hand, had the highest mycelial growth when compared to the growths of the other isolates (Table 1).

At a temperature of 25 °C, the isolates 2, 4 and 8, showing the smallest growths, isolates 1, 3, 6, 7, 9, presented median mycelial growth. And isolates 5 and 10, obtained the highest mycelial growths when compared to the growths of the other isolates (Table 1).

Temperature of 35 °C, isolates 2 and 4, showing the lowest growth, isolates 1, 3 and 5, obtained mediating growth. And isolates from 6 to 10, obtained the highest mycelial growth of *F*. spp. when compared to the others (Table 1).

For the continuous dark regime, at a temperatures of 15, 25 and 35 °C, there were significants differences in relation to the mycelial growth of *F*. spp., at 15 °C, the isolates 1, 2, 3 and 4, showing the lowest mycelial growth, and isolates from 5 to 10, presented median growth when compared too much (Table 1).

At a temperature of 25 °C, the isolates 1 and 2, showed the lowest growth, while isolates 5 and 10, showed the highest growth. However, isolates 3, 4, 6, 7, 8 and 9, mycelial growth was mediating for this pathogen (Table 1).

Using the temperature of 35 °C, isolate 1 showed the lowest growth. For isolates 2, 3, 4 and 7, they presented mediated mycelial growth, while isolates 5, 6, 8, 9 and 10, showed greater growth in relation to all the isolates evaluated (Table 1).

For the regime of alternating light at a temperatures of 15 and 35 °C, there were significants differences in relation to the mycelial growth of *F*. spp., at 15 °C, the isolates 1, 2 and 8, showed less growth, isolates 3, 4, 6 and 9, presented mediated growth, and isolates 5 and 7, obtained the highest growth in relation to the other evaluated isolates (Table 1).

At a temperature of 25 °C, there were no significants differences in the behavior for the mycelial growth of *F*. spp., since all isolates from 1 to 10, obtained great performances in mycelial growth (Table 1).

At a temperature of 35 °C, isolates 1, 2 and 4, obtained the smallest growths, while 3, 5, 6, 7, 8, 9 and 10 showed median mycelial growth (Table 1)

Brazilian Journal of Development

Table 1. Mycelial growth (cm) of *Fusarium* spp. isolates, from pineapple production areas in three Northeast producing states, submitted to differents cultivations conditions (light and temperatures regimes) in Potato Dextrose Agar (PDA) culture medium.

Light of Regimes	Temperatures (°C)	Mycelial growth (cm)									
		Isolates									
		1	2	3	4	5	6	7	8	9	10
Continuous light	15	0.19 hBχ[1]	1.31 fBχ	3.08 eAβ	2.67 fAχ	3.69 eAχ	4.52dAβ	4.93dAβ	4.24dAβ	4.13dAβ	4.49dAβ
	25	2.73 dAχ	1.63 fBχ	3.07 eAβ	2.30 fAχ	9.00 aAα	3.71eAχ	4.39dAβ	3.76 fAχ	4.08dAβ	8.42aAα
	35	2.91 eAχ	1.43 fBχ	3.25 eAβ	2.64 fAχ	3.65 eAχ	4.71dAβ	4.98dAβ	4.93dAβ	4.37dAβ	4.33dAβ
Continuous Dark	15	0.82 gBχ	2.83 fAχ	2.29 fAχ	2.40 fAχ	4.42 dAβ	4.26dAβ	4.97 dAβ	3.15dAα	4.82dAβ	3.96dAχ
	25	2.52 fAχ	2.26 fAχ	3.32 eAβ	3.36 eAβ	9.06 aAα	3.00eAβ	3.34 eAβ	4.91eAβ	4.56eAβ	8.88aAα
	35	2.96 cAχ	3.85 eAβ	3.97 eAχ	3.14 eAβ	4.65 dAβ	4.71dAβ	3.35 eAβ	4.58bAβ	4.72dAβ	4.79dAβ
Alternating light	15	0.70 fAχ	1.58 fBχ	3.77 eAχ	3.49 eAχ	4.33 dAβ	3.38eAβ	4.55 dAβ	2.80 fAχ	3.32eAχ	3.89eAχ
	25	8.47 aAα	8.00 aAα	8.20 aAα	10.64 aAα	9.00 aAα	8.23aAα	8.97 aAα	8.92aAα	9.00aAα	9.00aAα
	35	2.61 fAχ	2.31 fAχ	3.60 eAχ	2.31 fAχ	4.25 eAβ	3.26eAβ	3.58 eAβ	3.42eAχ	3.10eAχ	331 eAχ

1 Means followed by the same letter do not differ from each other by the Scott-Knott test ($p \leq 0.05$), being lower case for isolates, upper case for temperatures and the Greek letter for light regimes.

Behavior similar to the present study was verified by Garcia *et al.* (2015) when they evaluated the in vitro study of *F.* spp. under different conditions of temperature and light and observed that alternating light and a temperature of 25 °C favored the highest mycelial growth averages (4.40 cm) in *F.* spp. evaluated. Silva; Teixeira *et al.* (2012), studying the mycelial growth of *Fusarium solani* in different culture média and light regimes, also found that alternating light, at a temperature of 25 °C in PDA medium, induced the largest mycelial growth, obtaining an average of 6.73 cm.

Costa *et al.* (2009) when analyzing mycelial growth of *Fusarium subglutinans* f. sp. pineapples at different temperatures and photoperiods, found that the temperature of 25 °C under a photoperiod of 12 hours (alternating light) were the ideal conditions for the greater growth of this pathogen.

Evaluating the continuous light regime, at temperatures of 15, 25 and 35 °C, there were significant differences in relation to the behavior of *Fusarium* spp. as to the production of conidia, since at a temperature of 15 °C, isolates 1, 2, 3, 4, 7, 8 and 9, showed low production, while isolates 5, 6 and 10, obtained the highest conidia production (x 10^5/mL) (Table 2).

At 25 °C, only isolates 4 and 9 had the lowest yields, whereas isolates 1, 2, 3, 6, 7, 8, had average yields, while isolates 5 and 10 performed the highest conidia yields (x 10^5/mL) (Table 2).

For the temperature of 35 °C, isolates 4 and 9 obtained the lowest yields and isolates 1, 2, 3, 5, 6, 7, 8 and 10, presented median conidia yields (x 10^5/mL) (Table 2).

Using the continuous dark regime, at temperatures of 15, 25 and 35 °C, there were significant differences in relation to the behavior of *Fusarium* spp. as for the production of conidia, the temperature of 15 °C, isolates 1, 2, 3, 4, 6, 7, 8, 9 and 10, obtained the lowest yields and only isolate 5 presented median production of conidia (x 10^5/mL) (Table 2).

At a temperature of 25 °C, isolates 3, 4 and 9 showed the lowest yields, whereas isolates 1, 2, 6 and 7, obtained average production of conidia and isolates 5 and 10 stood out for their high yields (x 10^5/mL) (Table 2).

At a temperature of 35 °C, isolates 8 and 9 showed low production, while the other isolates 1, 2, 3, 4, 5, 6, 7 and 10, showed median production of conidia (x 10^5/mL) (Table 2).

Evaluating the alternating light regime, at temperatures of 15 and 35 °C, there were significant differences in relation to the behavior of *Fusarium* spp. as for the production of conidia, at a temperature of 15 °C, isolates 1, 2, 3, 4, 7, 8 and 9, presented low conidia production, and only isolates 5, 6 and 10, obtained median production of conidia (x 10^5/mL) (Table 2).

At a temperature of 25 °C, no significant differences were observed in relation to the behavior of *Fusarium* spp. as to the production of conidia, isolates from 1 to 10, obtained high conidia production (x 10^5/mL) (Table 2).

For the temperature of 35 °C, isolates 1, 2, 3, 4 and 7, presented median production, while isolates 5, 6, 8, 9 and 10, stood out for the high production of conidia (x 10^5/mL) (Table 2).

Garcia *et al.* (2015) evaluating the in vitro behavior of *F. guttiforme* under different conditions of temperature and light, concluded that the alternating light regime and the temperature of 25 °C favored the greater production of conidia that varied between 12.91 and 16.91 x 10^5 conidia/mL. Silva; Teixeira *et al*. (2012) evaluating the mycelial growth of *F. solani* in different culture média and light regimes, also found that alternating light, at a temperature of 25 °C in PDA medium, induced the highest production of conidia with 6.17 to 8.05 x 10^3 conidia/mL.

Brazilian Journal of Development

Table 2. Conidia production (x 10^5/mL) of *Fusarium* spp. isolates, from pineapple production areas in three Northeast producing states, submitted to differents cultivations conditions (light and temperatures regimes) in Potato Dextrose Agar (PDA) culture medium.

		(x 10^5 conidia/mL)									
Light of Regimes	Temperatures (°C)	Isolates									
		1	2	3	4	5	6	7	8	9	10
Continuous light	15	6.66 dBχ	1.55 dBχ	0.95 dBχ	0.26 dBχ	11.99 cCχ	13.83 cCχ	1.55 dBχ	095 dBχ	0.26 dBχ	11.99 cCχ
	25	26.42 bBβ	21.99 bBβ	13.69 bBχ	3.05 dBχ	73.05 aAα	18.26bBχ	28.24bBβ	15.12bBχ	0.11 dBχ	86.24 aAα
	35	14.14 bBχ	14.14 bBχ	10.08 bBχ	3.23 cCχ	32.84 bBβ	12.86bBχ	14.14bBχ	10.08bBχ	3.23 cCχ	32.84 bBβ
Continuous dark	15	5.93 dBχ	4.97 dBχ	1.77 dBχ	0.13 dBχ	18.18 bBχ	5.39 dBχ	4.97 dBχ	1.44 dBχ	8.56 dBχ	5.93 dBχ
	25	25.68 bBβ	28.24 bBβ	15.12 cCχ	14.44 cCχ	76.24 aAα	26.35bBβ	22.24bBβ	4.57 dBχ	16.60 cCχ	85.68 aAα
	35	28.00 bBβ	30.43 bBβ	23.48 bBβ	26.91 bBβ	29.69 bBβ	28.00bBβ	24.16bBβ	6.08 dBχ	14.29 cCχ	28.00 bBβ
Alternating light	15	4.79 dBχ	4.40 dBχ	5.90 dBχ	0.91 dBχ	9.60 cCχ	14.79 cCχ	6.06 dBχ	4.98 dBχ	0.92 dBχ	14.79 cCχ
	25	78.14 aAα	73.28 aAα	82.11 aAα	81.85 aAα	288.93 aAα	94.28aAα	84.50aAα	80.32aAα	90.60aAα	106.28 aAα
	35	24.28 bBβ	28.83 bBβ	49.86 bBβ	25.56 bBβ	73.24 aAα	80.81aAα	28.75bBβ	81.36aAα	82.56aAα	72.14 aAα

1 Means followed by the same letter do not differ from each other by the Scott-Knott test ($p \leq 0.05$), being lower case for isolates, upper case for temperatures and the Greek letter for light regimes.

According to Maia *et al.* (2015) physical agents can induce or inhibit vegetative and reproductive development in most fungi, with temperature being one of the most important factors. According to Pinheiro *et al.* (2012) for each fungal species requires an ideal temperature range for sporulation, which can be reduced under low temperatures and increased as the temperature rises, until reaching a maximum point in the sporulation. Inferring about the results observed in the present study, it was proved that the temperature in the ranges of 25 to 35 °C are favorable to the high rates of mycelial growth and production of conidia in several *Fusarium* species.

It can be seen that *F.* spp. poses a greater threat to the pineapple crop due mainly to climatic factors, because according to Koppen; Geiger (1928), the average temperature of the city of Pombos in the state of Pernambuco is 23.8 °C, with an average annual precipitation of 983 mm and humidity

relative air temperature around 80%, while in Touros, a municipality located in Rio Grande do Norte-Brazil, the average temperature is 26.1 °C and the average annual precipitation is 1.027 mm and relative humidity around 90%.

This observation justifies the development of *F*. spp., since in both places there are fields of pineapple production and provide an optimum temperature, precipitation and relative humidity environment. These factors influence the high mycelial growth and sporulation, mainly due to the modulation in the production of proteins and enzymes responsible for the maintenance of the fungal cell, in addition to the influence on cell multiplication (MORAIS *et al*., 2012).

At temperatures below 15 °C, there is generally no stimulus in the production of mycelium and sporulation, what may have occurred and decrease in the pathogen's metabolism observed.

On the other hand, Botelho; Monteiro (2011), observed that at temperatures above 30 °C, there is a prevented growth of mycelial and in the production of spores, since some essential enzymes can denature or have their formation altered, not resuming metabolic activity, which can drastically reduce sporulation.

There is no consensus on the effect of light under the biochemical mechanism of the formation of *Fusarium* conidiophores. However, for sporulation, light regimes are important factors, since they can influence sporulation in several species of phytopathogenic fungi (DEMARTELAERE *et al*., 2018).

In the present study, alternating light stimulated the greatest mycelial growth and sporulation of *F*. spp. evaluated. Thus, these conditions of the cultivation environment represented the ideal for the mass production of spores of this pathogen (MAIA *et al.*, 2015).

Light is also an important physical agent that influences the activation of key enzymes, responsible for the synthesis of compounds that are present in culture media, such as the PDA medium, used in the present work, which is rich in sources of nitrogen, phosphorus, salts minerals and high levels of sugar in its constitution, being essential elements for the increase in the production of mycelium and conidiophores to occur in several species of *Fusarium* (STANGARLIN *et al*., 2011).

To develop fusariosis control techniques, it is necessary to know the physiological, morphological, genetic and environmental characteristics of the pathogen in question. In this way, in vitro culture assesses the best conditions for the growth of *F*. spp. through its development in temperatures, incubation times, culture media and ideal photoperiods (MAIA *et al.*, 2015), allowing the *in vitro* multiplication of *F*. spp. on a large scale, in order to use isolates in inoculations to identify resistant materials, since planting cultivars with this characteristic has been the most economical, environmentally correct and effective method to control fusariosis (CAETANO *et al*., 2015).

Under certain cultivation conditions, they were important for the reproduction of *F.* spp. in the pineapple. However, little is known about the influence of these variations in the environment on the genetic variability of the pathogen that is known to cause serious damage to pineapple growers, as it reduces the quality and productivity of fruits in the producing regions of Brazil (TOFOLI *et al*., 2013). However, there is a need for research of this nature, using methodologies in controlled environments for later use of the information acquired with better and more appropriate management of fusariosis in the pineapple culture.

The effectiveness of management strategies for disease control is clearly dependent on understanding the pathogen and its population dynamics (COSTA *et al*., 2010). Therefore, studies on variability in fungal populations can be an important research tool. From an evolutionary point of view, the genetic variability of populations is important to determine the potential for adaptation of the organism to different environmental conditions and from an epidemiological point of view, pathogenic variability has direct implications for disease management (SOUZA, 2015). Thus, it becomes clear the importance of information about this pathosystem, as well as, the management of fusariosis in pineapple culture in several producing regions in Northeast Brazil.

4 CONCLUSIONS

The recommended culture conditions for mycelial growth and multiplication of *Fusarium* spp. are the alternating light regime and temperature of 25 °C.

REFERENCES

1. Aquije, G. M. F. V.; Zorval, P.B.; Buss, D. S.; J. A.; Ventura, P. M.; Fernandes, A.; Fernandes, A. Cell wall alterations in the leaves of fusariosis-resistant and susceptible pineapple cultivars. **Plant Cell Reports**, 2010: 29(1): 1109-1117.

2. Bisby, F. A.; Roskov, Y. R.; Ruggiero, M. A.; Orrell, T. M.; Paglinawan, L. E.; Brewer, P. W.; Bailly, N.; Van-Hertum, J. **Species Fungorum Reading**, 2006. 766 p.

3. Botelho, A. A. A.; Monteiro, A. C. Sensibilidade de fungos entomopatogênicos a agroquímicos usados no manejo da cana-de-açúcar. **Bragantia**, 2011; 70(1): 361-369.

4. Caetano, L. C. S.; Ventura, J. A.; Balbino, J. M. S. Comportamento de genótipos de abacaxizeiro resistentes à fusariose em comparação a cultivares comerciais suscetíveis. **Revista Brasileira de Fruticicultura**, 2015; 37(1): 404-409.

5. Costa, R.V.; Cota, L.V.; Pereira, D. F.; Silva, D. D.; Guimarães, P. E.; Guimarães, L. J. M.; Paretoni, S. N.; Pacheco, C.A.P. Desenvolvimento de metodologia para inoculação de *Colletotrichum graminicola* em colmo de milho. Sete Lagoas: Embrapa Milho e Sorgo, (**Circular Técnica, 139**). 2010. 6 p.

6. Costa, N. F. P.; Ferreira, I. C. P. V.; Aquino, C. F.; Araújo, A.V.; Sales, N. L. P. Crescimento micelial de *Fusarium subglutinans* f sp. *ananas* em diferentes temperaturas. Instituto de Ciências agrárias/UFMG, Montes Claros, MG. In: **Congresso Brasileiro de Fitopatologia 42**. Pelotas, 2009.

7. Cruz, M. F. A.; Prestes, A. M.; Maciel, J. L. N. Esporulação de *Pyricularia grisea* em diferentes meios de cultura e regimes de luz. **Ciência Rural**, 2009; 9(1): 1562-1564.

8. Demartelaere, A. C. F.; Nascimento, L. C.; Marinho, C. O.; Nunes, M. C.; Abraão, P. C.; Gomes, R. S. S.; Almeida, L.; Oliveira, M. D. M ;Porcino, M. M.; Silva, C. V.; Souza, W. C. O. Diversity among Isolates of the Tangerine Pathotype of *Alternaria alternata*. **American Journal of Plant Science**, 2018; 9(1): 196-215.

9. Demartelaere, A. C. F. Mancha Marrom de Alternaria em Tangerineira 'Dancy': Aspectos Morfofisiológicos, Variabilidade Genética e Indução de Resistência, **Revista Cultivar**, 2015. 15 p.

10. Garcia, W. M.; Krause, W.; Araújo, D.V.; Silva, C. A.; Miranda, A.F. Comportamento *in vitro* do agente etiológico da fusariose e avaliação de métodos de inoculação em abacaxizeiro. Revista Caatinga, 2015; 28(1): 263 – 268.

11. ***IBGE.*** *Levantamento sistemático da produção agrícola (**2019). Rio de Janeiro: LSPA. Disponível** em:< http://www.sidra.ibge.gov.br/cgi-bin/prtabl>**. Acesso em: 15** de Fev. de 2020.*

12. Koppen, W.; Geiger, R. Klimate der Erde. Gotha: **Verlag Justus Perthes**. Wall-map. 1928.

13. Leslie, J. F.; Summerell, B. A.; Bullock, S. The fusarium laboratory manual. Wiley-Blackwell, **Turner Published Online**, 2006. 388 p.

14. Lima, V. P. de; Reinhardt, D. H.; Costa, J.A. Desbaste de mudas tipo filhote do abacaxi cv. Pérola - 1. Produção e qualidade do fruto. **Revista Brasileira de Fruticultura**, 2001; 23(1): 634-638.

15. Maia, A. J.; Schwan-Estrada, K. R. F.; Faria, C. M. D. R.; Santos, L. A.; Oliveira, J. B. S.; Santos, R.C. Produção de esporos e efeito da temperatura e luminosidade sobre germinação e infecção de *Pseudocercospora vitis* em videira. **Summa Phytopathologica**, 2015; 41(1): 287-291.

16. Martelleto, L. A. P. Incitante da fusariose do abacaxizeiro (*Ananas comosus* (L.) Merril) e sobre o efeito da temperatura ambiente no seu desenvolvimento, 1995. 30 p.

17. Menezes, M.; Assis, S. M. P. **Guia Prático para fungos fitopatogênicos**. 2ª edição, imprensa universitária, UFRPE, Recife-PE. 2004.

18. Morais, T. P.; Luz, J. M. Q.; Silva, S. M.; Resende, R. F.; Silva, A.S. Aplicações da cultura de tecidos em plantas medicinais. **Revista Brasileira de Plantas Medicinais**, 2012; 14(1): 110-121.

19. Niremberg, H. I.; O'donnell, K. New *Fusarium* species and combinations within the *Gibberella fujikuroi* species complex. **Micologia**, 1998.103 p.

20. O'donnell, K.; Kistler; H. C.; Cigelnik, E.; Ploetz, R. C. Multiple evolutionary origins of the fungus causing Panama disease of banana: concordant evidence from nuclear and mitochondrial gene genealogies. **Proceedings of the National Academy of Sciences**, 1998; 95(1): 2044–2049.

21. Oliveira, J. A. **Efeito do tratamento fungicida em sementes e no controle de tombamento de plântulas de pepino** (*Cucumis sativus* L.) **e pimentão** (*Capsicum annuum* L.). 1991. 111 p. Dissertação (Mestrado em Fitossanidade) - Escola Superior de Agricultura de Lavras, Lavras, 1991.

22. Paulino, J. F. C.; Almeida, C. P.; Bueno, C. J.; Benchimol-Reis, L. L. Índice relativo de clorofila na avaliação da murcha de Fusarium em feijoeiro. **Brazilian Journal of Development**, 2019; 5(11): 24635-24642.

23. Poltronieri, T. P. S.; Azevedo, L. A. S.; Silva, D. E. M. Effect of temperature on mycelial growth and conidial production and germination for *Colletotrichum gloeosporioides* isolated from juçara palm fruits (*Euterpe edulis* Mart). **Summa Phytopathologica**, 2013; 39(1): 281-285.

24. Pinheiro, G. S.; Angelotti, F.; Costa, N. D.; Santana, C.V. da S.; Rodrigues, D. R. Impacto de alterações da temperatura sobre o crescimento e esporulação de *Alternaria porri*. **52º Congresso Brasileiro de** Olericultura, 2012. 2p.

25. R Development Core Team (2011) R: a language and environment for statistical computing. **R Foundation for Statistical Computing**. Disponível em:< http://www.R-project.org/>. Acesso em: 15 de Jun. 2020.

26. Silva, J. L.; Teixeira, R. N. V. Esporulação e crescimento micelial de *Fusarium solani* em diferentes meios de cultura e regimes de luminosidade. **Revista Agroambiente**, 2012; 6(1): 47-52.

27. Souza, W. C. O.; Nascimento, L. C.; Oliveira, M. D. M.; Porcino, M. M.; Silva, H. A. O. Genetic diversity of *Fusarium* spp. in pineapple 'Pérola' cultivar. **European Journal Plant Pathology**, 2017; 149(1): 1-16.

28. Souza, D. C. L. Técnicas moleculares para caracterização e conservação de plantas medicinais e aromáticas: uma revisão. **Revista Brasileira de Plantas Medicinais**, 2015; 17(3): 495-503.

29. Stangarlin, J. R.; Kuhn, O. J.; Toledo, M.V.; Portz, R. L.; Schwan-Estrada, K. R. F.; Pascholati, S. F. A defesa vegetal contra fitopatógenos. **Revista Scientia Agrária**, 2011; 10(1): 18-46.

30. Tofoli, J. G.; Melo, P. C. T.; Domingues, R. J.; Ferrari, J. T. Potato late blight and early blight: importancy, characteristics and sustainable management. **Revista Arquivos do Instituto Biológico**, 2013; 75(1): 33-40.